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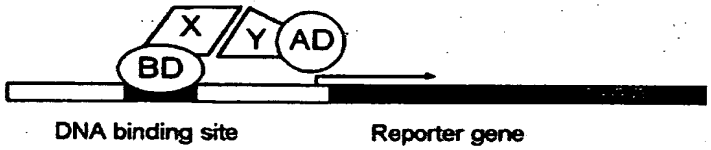
Topic 23.11

The Two-Hybrid System

Many cellular proteins interact with a variety of other proteins to perform their physiological functions. Identification of interacting proteins can be accomplished by biochemical assays, such as co-purification of a complex, but these require relatively stable interactions and the ability to identify components of the complex immunologically or based on amino acid sequence and composition. Alternatively, the two-hybrid assay is a genetic approach that takes advantage of the modular nature of proteins to identify interacting domains. Although there are several variations, in all cases two fusion proteins are co-expressed in a cell line that will activate expression of a reporter gene if the fusion proteins interact. For example, the yeast transcription factor GAL4 can be separated into distinct DNA binding (BD) and transcription activation (AD) domains. If hybrid genes are constructed such that they encode fusion proteins BD-protein X and AD-protein Y, interactions between proteins X and Y will bring the GAL4 domains close enough to reconstitute a functional transcription factor that can trans-activate a GAL4-responsive promoter (Web Figure 23.11.A). The cell lines often contain several GAL4-responsive promoter-reporter fusions, whose activity can be scored either qualitatively or quantitatively. Qualitative scoring (+/- growth) makes it possible to screen a cDNA library for fusions encoding interacting proteins by requiring expression of a gene whose activity can complement an auxotrophy. Quantitative scoring (e.g. β-galactosidase activity from a *lacZ* reporter) permits comparisons of the relative strength of interactions among a variety of protein pairs (Drees 1999; Fashena et al. 2000; Ito et al. 2001).



Web Figure 23.11.A Two fusion proteins containing the GAL4 DNA binding (BD) and transcription activation (AD) domains can reconstitute a functional transcription factor that can trans-activate a GAL4-responsive promoter if the interactions between the two proteins, X and Y, will bring the GAL4 domains close enough together. **(Click image to enlarge.)**

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